OPHTHALMIC DEVICE COMPRISING A HOLOGRAPHIC SENSOR

Field of the Invention

This invention relates to an ophthalmic device comprising a holographic sensor.

Background to the Invention

Ophthalmic devices, for example contact lenses, comprising holographic elements are known. Typically, a holographic element is used to focus incoming light. The holographic element may have a programmed activating angle providing two or more optical powers. The use of a holographic element allows the user to see clear and unimpaired images, thereby overcoming many of the shortfalls of traditional simultaneous vision and translating lenses. Holographic optical inserts are described, for example, in WO-A-99/34239, WO-A-99/34244, WO-A-02/054137 and WO-A-99/34248.

The need for minimally invasive, easy-to-use glucose sensors has motivated the investigation of numerous approaches. One particular area of interest is ophthalmic glucose sensors, i.e. those for the detection of glucose in the eye. The levels of glucose in the eye are known to correlate with those in the blood, and thus blood levels of glucose can be monitored indirectly by measuring the levels in an ocular fluid such as tears.

US-A-2003/0027240 describes an ocular insert for the detection of glucose. The insert comprises a polymerised crystalline colloidal array (PCCA) which is polymerised in a hydrogel, and a molecular recognition component capable of responding to glucose. The array has a lattice spacing that changes when the volume of the hydrogel changes, causing the diffracted wavelength of the array to change.

WO-A-95/26499 discloses a holographic sensor, based on a volume hologram. This sensor comprises an analyte-sensitive matrix having an optical transducing structure disposed throughout its volume. Because of this physical arrangement of the transducer, the optical signal generated by the sensor is very sensitive to volume changes or structural rearrangements taking place in the analyte-sensitive matrix as a result of interaction or reaction with the analyte. For example, a sensor comprising a gelatin-based holographic medium may be

used to detect trypsin. Trypsin acts on the gelatin medium, irreversibly destroying the integrity of the holographic support medium. Holographic sensors may also be used to detect changes in, for example, pH.

Although sensors of the kind described in US-A-2003/0027240 may be used to detect levels of glucose in the eye, there remains the need for ophthalmic sensors which allow for accurate, real-time detection of analytes such as glucose.

Summary of the Invention

The present invention is based on the realisation that holographic sensing technology, when incorporated into a contact lens or other ophthalmic device, provides an accurate yet minimally invasive method of detection of an ocular analyte. Such sensing technology may allow for the continuous, *real-time* sensing of glucose or other carbohydrates. The invention thus may improve the lives of patients having diabetes and decrease such patients' risk of developing hypoglycemia or hyperglycemia.

A first aspect of the invention is an ophthalmic device which comprises a holographic element comprising a medium and, disposed therein, a hologram, wherein an optical characteristic of the element changes as a result of a variation of a physical property of the medium, and wherein the variation arises as a result of interaction between the medium and an analyte present in an ocular fluid. The device may be a contact lens or an ocular implant.

Another aspect of the invention is a method of detection of an analyte in an ocular fluid, the method comprising detecting any change of the optical characteristic of the holographic element of a device of the invention with the fluid, in the eye.

Another aspect of the invention is a method for the production of a device of the invention which comprises contacting the holographic element with a contact lens, wherein the contacted regions of the element and the lens are cross-linkable; and cross-linking said regions. Preferably, at least one of the contacted regions comprises PVA, more preferably Nelfilcon.

The invention may be used for the detection of ocular analytes such as glucose or lactate. The interaction is preferably reversible so that both the

interaction and reverse interaction can occur, allowing the analyte to be continuously detected, preferably in real time. The interaction is preferably a chemical reaction.

Description of the Invention

The term "glucose" as used herein refers to the known cyclic and linear forms of glucose.

The term "ophthalmic device" as used herein refers to contact lenses (both hard and soft), corneal onlays, implantable ophthalmic devices and the like.

The term "contact lens" as used herein refers to any hard or soft lens used on the eye or ocular vicinity for vision correction, diagnosis, sample collection, drug delivery, wound healing, cosmetic appearance or other ophthalmic application. The lens may be a daily-disposable, daily-wear or extended-wear lens.

The term "implantable ophthalmic device" as used herein refers to an ophthalmic device which is used in, on or about the eye or ocular vicinity. Such devices include intraocular lenses, subconjunctival lenses, intracorneal lenses, and shunts/implants (e.g. a stent or glaucoma shunt) that can rest in the cul de sac of an eye.

In a preferred embodiment, the insert is in the form of a contact lens. The lens may be manufactured using any suitable material known in the art. The lens material may be formed by the polymerisation of one or more monomers and optionally one or more prepolymers. The material may comprise a photoinitiator, visibility tinting agent, UV-blocking agent and/or a photosensitiser.

A preferred group of lens materials are prepolymers which are water-soluble and/or meltable. It is preferred that the material comprises one or more prepolymers which are in a substantially pure form (e.g. purified by ultrafiltration). Preferred prepolymers include water-soluble crosslinkable poly(vinyl alcohol) prepolymers (as described in US5583163 and US6303687); a water-soluble vinyl group-terminated polyurethane, obtainable by reacting an isocyanate-capped polyurethane with an ethylenically unsaturated amine (primary or secondary amine) or an ethylenically unsaturated monohydroxy

compound, wherein the isocyanate-capped polyurethane can be a copolymerisation product of at least one polyalkylene glycol, a compound containing at least 2 hydroxyl groups, and at least one compound with two or more isocyanate groups; derivatives of a polyvinyl alcohol, polyethyleneimine or polyvinylamine (see, for example, US5849841); a water-soluble crosslinkable polyurea prepolymer as described in US6479587; crosslinkable polyacrylamide; crosslinkable statistical copolymers of vinyl lactam, MMA and a comonomer, as disclosed in EP-A-0655470 and US5712356; crosslinkable copolymers of vinyl lactam, vinyl acetate and vinyl alcohol, as disclosed in EP-A-0712867 and US5665840; polyether-polyester copolymers with crosslinkable side chains, as disclosed in EP-A-0932635; branched polyalkylene glycol-urethane prepolymers, as disclosed in EP-A-0958315 and US6165408; polyalkylene glycol-tetra(meth)acrylate prepolymers, as disclosed in EP-A-0961941 and US6221303; and crosslinkable polyallylamine gluconolactone prepolymers, as disclosed in WO-A-00/31150.

The lens may comprise a hydrogel material. Typically, hydrogel materials are polymeric materials which are capable of absorbing at least 10% by weight of water when fully hydrated. Hydrogel materials include poly(vinyl alcohol) (PVA), modified PVA (e.g. nelfilcon A), poly(hydroxyethyl methacrylate), poly(vinyl pyrrolidone), PVA with a poly(carboxylic acid) (e.g. carbopol), poly(ethylene glycol), polyacrylamide, polymethacrylamide, silicone-containing hydrogels, polyurethane, polyurea, and the like.

Alternatively, the device may be an implantable ophthalmic device. Glucose levels in tears may be much lower than blood glucose levels. With an implantable ophthalmic sensor, one can monitor glucose levels in aqueous humor or interstitial fluid, where glucose levels can be much higher than glucose levels in tears. Preferably, the device is in the form of a subconjunctive implant, intracorneal lens, stent or glaucoma shunt.

The holographic support medium is one in which a hologram can be made and which is capable of exhibiting one or more of the properties of the sensitive mechanisms described herein. The hologram may be disposed on or in, part of or throughout the bulk of the volume of the support medium. Particularly in the

case of a contact lens, the support medium may be an integral part of the device, e.g. the body of a lens may itself comprise or form a holographic support medium.

The support medium preferably comprises a native or modified matrix with viscoelastic properties which alter as a result of an interaction with an analyte species. For example, the matrix may be formed from the copolymerisation of (meth)acrylamide and/or (meth)acrylate-derived comonomers. In particular, the monomer HEMA (hydroxyethyl methacrylate) is readily polymerisable and cross-linkable. PolyHEMA is a versatile support material since it is swellable, hydrophilic and widely biocompatible.

A device in the form of a contact lens is preferably obtained by forming a holographic element and then embedding the element into a contact lens. For example, a contact lens sensor may be obtained using the following protocol:

(a) forming a polymeric holographic sensor (e.g. using phenylboronate ligands) on a glass slide or similar surface;

(b) coating a layer of polyvinylalcohol (PVA), preferably "Nelfilcon", onto the surface of the sensor, with subsequent cross-linking of the layer;

(c) extracting any toxic components from the coated sensor (e.g. using 1:1 mixture of methanol:water overnight at 40°C), followed by autoclaving;

(d) removing the sensor from the slide and cutting from it a disc of about 4mm diameter; and

(e) inserting a disc into a contact lens mould containing a contact lens and PVA, preferably Nelfilcon, then cross-linking and autoclaving the components to form the finished lens.

A holographic sensor of the type used in the invention generally comprises a medium and, disposed throughout the volume of the medium, a hologram. The support medium may interact with an analyte resulting in a variation of a physical property of the medium. This variation induces a change in an optical characteristic of the holographic element, such as its polarisability, reflectance, refractance or absorbance. If any change occurs whilst the

hologram is being replayed by incident broad band, non-ionising electromagnetic radiation, then a colour or intensity change, for example, may be observed.

The sensor may be prepared according to the methods disclosed in WO-A-95/26499, WO-A-99/63408 and WO-A-03/087789. The contents of these specifications are incorporated herein by reference.

More than one hologram may be supported on, or in, a holographic element. Means may be provided to detect the or each variation in radiation emanating from the or each hologram, arising as a result of a variation in the or each optical characteristic. The holographic elements may be dimensioned and arranged so as to sense two independent events/species and to affect, simultaneously, or otherwise, radiation in two different ways. Holographic elements may be provided in the form of an array.

An illuminating source of non-ionising radiation, for example visible light, may be used to observe variation(s) in the, or each, optical characteristic of the holographic element. The extent of interaction between the holographic medium and the analyte species is reflected in the degree of change of the physical property, which is detected as a variation in an optical characteristic, preferably a shift in wavelength of non-ionising radiation.

The property of the holographic element which varies may be its charge density, volume, shape, density, viscosity, strength, hardness, charge, hydrophobicity, swellability, integrity, cross-link density or any other physical property. Variation of the or each physical property, in turn, causes a variation of an optical characteristic, such as the polarisability, reflectance, refractance or absorbance of the holographic element.

There are a number of basic ways to change a physical property, and thus vary an optical characteristic. The physical property that varies is preferably the volume of the support medium and, in turn, the spacing of the holographic fringes of the holographic element. This variation may be achieved by incorporating specific groups into the support matrix, where these groups undergo a change in, for example, conformation, charge or the degree of cross-linking upon interaction with the analyte, and cause an expansion or contraction of the support medium. An example of such a group is the specific binding

conjugate of an analyte species. Another variation is in the active water, solvent or charge content of the support medium. In this case, the holographic support medium is preferably in the form of a gel.

Analyte molecules that can react with at least two functional groups in the element may form a reversible cross-link between separate parts of the support matrix, thereby altering the visco-elastic properties of the support matrix. Consequently, if present within a solvent-containing environment, and the support matrix changes, the support matrix contracts and the separation of the fringes is reduced. Specificity may be provided by ensuring that specific binding sites are provided within the medium.

The support medium may comprise a receptor which is capable of binding or interacting specifically with the analyte. Suitable receptors include antibodies, lectins, hormone receptors, drug receptors, enzymes, aptamers, nucleic acids, nucleic acid analogues, and fragments thereof.

A receptor may be incorporated into a support medium using any suitable method known in the art. For example, a prepolymer and receptor may comprise matching functional groups; the two components can then be covalently linked with one another. Alternatively, a receptor may be incorporated in a vinylic monomer which a component of the lens-forming material.

One parameter determining the response of the system is the extent of cross-linking. The number of cross-linking points due to polymerisation of monomers should not be so great that complex formation between polymer and analyte-binding groups is relatively low, since the polymer film may become too rigid. This may inhibit the swelling of the support medium.

By way of example of a glucose sensor, a hydrogel-based hologram may have a support medium comprising pendant glucose groups and a lectin, preferably concanavalin A (con A). The lectin binds to the pendant glucose groups and acts as a cross-linker in the polymer structure. In the presence of freely diffusible glucose, the extent of cross-linking will decrease as glucose in solution displaces polymer-attached glucose from the binding sites on the lectin, resulting in swelling of the polymer. Volume changes in hydrogel films containing pendant glucose groups and con A can be observed using a reflection hologram.

A volume change in the hydrogel alters the fringe separation of the holographic structure and can be followed as a shift in the peak wavelength of the spectral reflected response.

Water-based systems are preferred in such a holographic sensor, since they protect the lectin from exposure to organic solvents. Examples of suitable glucose components are high molecular weight dextran, and the monomers allylglucoside and 2-glucosyloxyethyl methacrylate (GEMA). Dextran, having no inherent polymerisable functionality, can be entrapped during the polymerisation of acrylamide-based monomers; allylglucoside and GEMA can be polymerised either individually or together with comonomers. The polymers are preferably prepared as thin films on glass supports.

A holographic glucose sensor may comprise any suitable glucose receptor, particularly one which allows a reversible change in a physical property of the support medium upon binding with glucose. For example, the support medium may comprise pendant boronic acid groups, such as phenylboronic acid or a derivative thereof. Two adjacent diol groups in glucose bind with a boronic acid group in a reversible condensation reaction. Thus in a holographic element, reaction of glucose with pendant phenylboronic acid groups causes an expansion of the support medium, due to the formation of boronate esters. Without wishing to be bound by theory, it is believed that the boronate esters are negatively charged and effect a Donan potential, causing water to partition into the support medium. This expansion is observed as a shift in the reflectance maxima to longer wavelengths. The sensing ability of boronic acid groups is strongly dependent on the molecular geometry and the aromatic species where the boronic acid group is present. Thus, glucose sensitive probes can be made with a variety of affinities, in the mM range for blood glucose, and in the μM range for tear glucose. Preferred boronic acid groups include those described in WO-A-04/081624.

Boronic acid compounds, in particular phenylboronic acid compounds, are versatile receptors since they may be used for the detection of a variety of carbohydrates. In physiological fluids, this lack of selectivity is not a problem because most sugars are found on glycoproteins and other macromolecular

structures, i.e. they are already bound and thus cannot bind to the boronic groups of the support medium. Glucose is the only sugar that is found free in relatively high concentration. Lactate (lactic acid), however, may pose a problem since it is an α-hydroxy acid which binds to boronic acid groups and is, in ocular fluids, generally present in a greater concentration than glucose.

The problem of lactate interference can be addressed by incorporating, in the device, a group which repels lactate. Lactate carries an overall negative charge in physiological fluids and thus, for example, the support medium may carry a group having a negative charge, the magnitude of which will be apparent to those skilled in the art. An example of such a group is glycolic acid, which can be incorporated into the support medium by the polymerisation of monomers including, for example, acrylamidoglycolic acid. The glycolic acid moiety competes with glucose and lactate for available phenylboronic acid sites however, since the moiety carries a negative charge, it repels lactate but not glucose. Alternatively, the boronic receptor may itself carry a substantial negative charge or polarisation, e.g. by coordinating the boron atom with suitable electron-donating groups. An example of such a boronic acid is 5-fluoro-2-methylacrylamidophenylboronic acid. Another option is to attach negatively charged groups to the phenyl group of a phenylboronate receptor. The surface of the holographic element or the device may be negatively charged, to reduce the effects of lactate interference.

A sensor can also be made more selective for glucose by incorporating pendant amine groups in the support medium. The nitrogen atom of the amine group may form an intramolecular bond with the boron atom, thereby promoting formation of the more reactive tetrahedral conformation about the boron atom.

The support medium may comprise one or more macrocyclic groups such as crown ethers, which reversibly bind a range of ionic species. Crown ethers are well known to reversibly bind Group I and Group II metal ions. Therefore a crown ether which is specific to an ionic analyte can be immobilised in the support medium and used to continuously monitor the presence of the analyte.

The following Examples illustrate the invention.

Example 1

A contact lens was produced according to the protocol described above. The embedded holographic element comprised 12% mol of 3-acrylamidophenylboronic acid, the synthesis of which is described in WO-A-04/081624.

The lens was placed into the eye of a human volunteer, who then ingested a 44g bolus of glucose. The response of the contact lens sensor was measured in terms of the shift in the wavelength of reflection. Blood glucose levels were also monitored directly using a conventional glucose sensor.

Fig. 1 shows the response of the contact lens sensor, Fig. 2 that of the blood glucose sensor. It is evident that the responses of the two sensors are similar, the peak level of glucose being absorbed at around t = 25 minutes.

Example 2

An experiment similar to that of Example 1 was performed, using an ophthalmic implant comprising the sensor. The support medium was coated with Nelfilcon (Cibavision).

The experiment was conducted on a rabbit, instead of a human volunteer, the device implanted subcutaneously just below the eye. The rabbit was then anaesthetised using an xylazine-based protocol which causes blood levels of glucose to rise to a level commonly seen in diabetic patients (see Cameron *et al*, Diabetes Technology & Therapeutics, 2001, 3, 201-207). The concentration of glucose was then monitored using the implant. Again, blood levels of glucose were also monitored directly.

Fig. 3 shows the response of the holographic implant, Fig. 4 that of the blood glucose sensor. As in Example 1, the responses of the two sensors are similar.

Example 3

A holographic support medium was formed by copolymerising 13 mol% 5-fluoro-2-methylacrylamidophenylboronic acid (the synthesised according to WO-A-04/081624) and 3% MBA in acrylamide. A holographic image was then recorded in the resulting medium and the sensor used to detect glucose in PBS at pH 7.4 and a temperature of 30°C. A similar experiment was performed to test the sensor's response to lactate.

The results are shown in Fig. 5. The improved selectivity to glucose over lactate is attributable to the oxygen- and nitrogen-based electron-donating groups coordinated to the boron atom of the phenylboronate receptor. These groups increase the negative change around the boron atom.

Example 4

A medium was obtained by polymerising 12 mol% 3-acrylamidophenylboronic acid, 12 mol% acrylamidoglycolic acid and 76 mol% acrylamide, using 2% (w/v) of 2-dimethoxy-2-phenyl-acetophenone (a free radical initiator) in dimethyl sulphoxide. A hologram was recorded in the medium, and the resulting sensor tested for its response to glucose and lactate.

The results are shown in Fig. 6. The presence of acrylamidoglycolic acid reduced the response of the sensor to the two analytes, as the negative charge of the acidic moiety causes a significant degree of swelling of the polymeric medium. However, the sensor was more responsive to glucose than lactate, because the glycolic acid component carries a negative charge which repels lactate, without significantly affecting glucose binding.

Example 5

A support medium was formed by copolymerising 11.9 mol% 3-acrylamidophenylboronic acid, 9.2 mol% N-[3-(dimethylamino)propyl]acrylamide, 2.9 mol% methylenebisacrylamide and 76 mol% acrylamide, by exposure to UV light for 1 hour. Silver ions, present in an acetic acid solution, were diffused into the medium, the acidic solution present to prevent "fogging" of the silver by the amine component. A hologram was recorded in the medium, and the resulting sensor tested for its response to glucose and lactate.

The results are shown in Fig. 7. The sensor was selective for glucose over lactate; the peak wavelength shift for lactate was only about 12% of that for glucose at the same concentration. Also, the wavelength shift is a negative shift for glucose, whereas the binding of lactate effects a positive shift. The presence of "background" (4mM) lactate had a negligible effect of the sensor's response to glucose.